**A 4-Week Repeated Oral Dose Toxicity Study of Project F in Cynomolgus Monkeys Followed by a 4-Week Recovery Period**

**11 SUMMARY**

Project F (Lot Number: 2848-18001) was suspended in vehicle (0.5 w/v% methylcellulose solution) and administered orally once daily for 4 weeks at dose levels of 0 (control), 3, 30, and 300 mg/kg (dose volume: 5 mL/kg) as free form to 4 male and 4 female cynomolgus monkeys (age at initiation of acclimation: 3 to 4 years) per group in order to investigate its toxicityand 3 males and 3 females in the highest dose group were set to evaluate the reversibility of toxic changes during a subsequent 4-week recovery period. For the control group, vehicle was administered in the same manner as test article. In the 300 mg/kg group, the dose level for females was decreased to 100 mg/kg from Day 15 of dosing because death or moribundity occurred. Thereafter, 1 male was found dead on Day 26 of dosing and 1 male was sacrificed due to moribundity on Day 28 of dosing. Three males and two females underwent terminal necropsy and 2 males and 2 females underwent recovery necropsy. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, electron microscopy, and toxicokinetics.

Three females at 300 mg/kg were sacrificed due to moribundity on Day 13 (1 female) and 14 (2 females) of dosing, 1 male was found dead on Day 26 of dosing, and 1 male was sacrificed due to moribundity on Day 28 of dosing. In these animals, vomiting was observed from Day 1 or 8 of dosing, decreased food consumption was noted in females from Week 1 of dosing and in males at Week 4 of dosing, and decreased body weight was also noted in all moribund animals. Before sacrifice or death, slight or moderate decreased spontaneous activity, abnormal position (lateral position), hypothermia, suppressed response to touch, and/or suppressed response to stimulation were observed. In additions, increased leukocyte count, neutrophil count, and monocyte count were noted in hematology; increased protein and decreased Cl excretion were noted in urinalysis; increased AST, ALT, total bilirubin, UN, and creatinine in blood chemistry. Prolonged QT and/or QTc were noted in electrocardiography. Enlargement of the liver was observed in gross pathology. Histopathological examination revealed granular degeneration of hepatocytes and granular hypertrophy of Kupffer cells in the liver and degeneration/necrosis and dilatation of the renal tubules and hyaline droplets in the proximal tubule in the kidneys. High liver and kidney weights were noted in the organ weights. Based on the the changes in urinalysis, blood chemistry, gross pathology, organ weights, and histopathological examination, it was suggested that the target organs are the liver and kidneys. The following changes were considered to be secondary changes related to deterioration in general condition: increased RBC, HGB, HCT, and MCHC; decreased MCV; and prolonged APTT in hematology; increased triglyceride, glucose, IP, and K and decreased Na and Cl in blood chemistry; bilateral enlargement of the adrenal glands with increased adrenal weights and small-sized thymus; and decreased lipids in the zona fasciculata or hypertrophy of the zona fasciculata in the adrenal glands in histopathology. The toxicity in the liver and/or kidney was considered to be a possible cause of death or moribund condition.

In surviving animals, no toxicologically significant changes were noted at 3 and 30 mg/kg.

In the highest dose groups (males: 300 mg/kg and females: 300 → 100 mg/kg), vomiting was observed in males and females from Day 1 of dosing and decreased food consumption in females from Week 2 of dosing and in 2 male at from Weeks 1 or 4 of dosing and decreased body weight were noted in males and females from Week 2 of dosing. A slight decrease in spontaneous activity was observed in 1 female on Day 14 and Day 15. The changes in clinical sings in females recovered after the reduction of the dose level. Prolongation of QT and QTc were noted in electrocardiography were noted. In clinical pathology, decreased RBC, HGB, HCT and/or increased reticulocyte ratio, increased leukocyte, neutrophil, and monocyte counts and/or increased fibrinogen were noted in hematology. In addition, increased protein and decreased Cl excretion in males were noted in urinalysis and increased AST, ALT, and total bilirubin were noted in blood chemistry. High liver and/or kidney weights were noted in the organ weights.

The following changes were observed in addition to those mentioned above: Histopathological examination revealed granular degeneration of the hepatocytes, granular hypertrophy of the Kupffer cells, and/or vacuolation of the hepatocytes in the liver. In electron microscopy, increase in electron-dense structures with irregular size was observed in the hepatocytes and an increase in secondary lysosomes in the Kupffer cells was observed in males and females in the high dose groups. Additionally, cytoplasmic vacuolation in the hepatocytes was observed. In the kidney, hyaline droplets in the proximal tubule (slight) and an increase in secondary lysosomes at the proximal tubular epithelium and cytoplasmic vacuolation in the proximal tubular epithelium were observed. The target organs were considered to be the liver and kidney. In surviving animals, secondary changes related to deterioration in general condition similar to those observed in the the dead or moribund animals were noted as follows: increased IP and decreased Ca and/or changes in electrolytes (Na, K, Cl); increased total protein and albumin; and histopathological changes in the thymus (decreased cellularity), and skin/subcutis (atrophy of adipose tissue).

No test article-related changes were noted in ophthalmology at any dose level.

In toxicokinetics, the tmax, Cmax, and AUC24 of PROJECT F are shown in the following table:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Dose Level  (mg/kg) | Day | tmax (h) | | Cmax (ng/mL) | | AUC24 (ng·h/mL) | |
| Male | Female | Male | Female | Male | Female |
| 3 | 1 | 1.50 | 1.00 | 144 | 131 | 534 | 426 |
| 14 | 1.00 | 0.875 | 161 | 136 | 553 | 457 |
| 28 | 0.875 | 0.875 | 410 | 115 | 1150 | 394 |
| 30 | 1 | 4.00 | 3.00 | 1910 | 2020 | 12900 | 11500 |
| 14 | 3.50 | 2.75 | 1950 | 2370 | 13300 | 17200 |
| 28 | 3.50 | 3.25 | 1870 | 1380 | 13400 | 9600 |
| 300\* | 1 | 4.57 | 3.86 | 6440 | 3790 | 78100 | 37500 |
| 14 | 5.71 | 4.00 | 7300 | 5990 | 94700 | 79500 |
| 28 | 5.60 | 4.00 | 6950 | 6140 | 95100 | 66600 |

\*: The females received 300 mg/kg (as free form) until Day 14 of dosing and 100 mg/kg from Day 15 of dosing.

In toxicokinetics, the Cmax and AUC24 generally increased with dose level. There were no apparent changes in the TK parameters after repeated dosing. There were no apparent sex differences in the TK parameters. In females, the dose level was reduced to 100 mg/kg from Day 15 of dosing, but there was no clear decrease in TK parameters.

The changes noted during the dosing period or at the end of the dosing period disappeared by the end of the recovery period, indicating that they were reversible.

It was concluded that, under the conditions of this study, the target organs of Project F were the kidney and liver. The no-observed-adverse-effect level (NOAEL) of Project F was 30 mg/kg/day as PROJECT F for males and females because granular degeneration of the hepatocytes in the liver in the highest dose group and moribund or dead animals were observed. The test article-related changes noted during the dosing period or at the end of dosing period recovered by Week 4 of the recovery period.